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# The Relationship of Gentamicyn Antibiotic Exposure To: *Escherichia coli* Bacteria Resistant to Antibiotic Gentamicyn and *Escherichia coli* ESBL In Vitro

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**ABSTRACT**

**Introduction:** The development of bacteria that have been resistant to antibiotics can complicate the treatment process. Either causes of antibiotic resistance is inappropriate use of antibiotics. *Gentamycin* is an aminoglycoside-derived antibiotic which its role is very significant for Gram-negative bacteria. Repeated use of *Gentamycin* antibiotics can cause changes the effectiveness of *Gentamycin* so that non ESBL-*Gentamycin* susceptible *Escherichia coli* will change into ESBL-*Gentamycin* resistant *Escherichia coli*. This study aims to prove that repeated exposure to *Gentamycin* in vitro will change non ESBL-*Gentamycin* susceptible *Escherichia coli* into ESBL *Gentamycin* resistant *Escherichia coli.*

**Methods:** This was an experimental study with 30 samples of non ESBL-*Gentamycin* susceptible *Escherichia coli* isolates identified from the Phoenix. Non ESBL-*Gentamycin* susceptible *Escherichia coli* was tested by giving exposure to *Gentamycin* for 14 days, then ESBL screening was tested by *Cefotaxime* exposure to the results of *Gentamycin* exposure.

**Result and Discussion:** There were 4 isolates of *Escherichia coli* which experienced changes in phenotype into *Gentamycin* resistant *Escherichia coli*. The rest of it still susceptible to *Gentamycin* on days 2, 4 and 10. Furthermore, the *Escherichia coli* isolates were both susceptible to *Gentamycin* and those that had phenotypic changes become resistant to *Gentamycin* after exposed to *Cefotaxime* as an ESBL screening. There are 8 (26.7%) isolates that are still susceptible to *Cefotaxime* and 18 (60%) isolates that have been transformed into ESBL-*Gentamycin* susceptible *Escherichia coli*. Isolates of 4 (13.3%) *Gentamycin*-resistant *Escherichia coli* are then exposed to *Cefotaxime* and obtained all isolates is resistant to *Cefotaxime*.

**Conclusion:** Repeated exposure of *Gentamycin* for 14 days in vitro was not significantly related to the phenotypic changes of non ESBL-*Gentamycin* susceptible *Escherichia coli* isolates into ESBL-*Gentamycin* resistant *Escherichia coli* (P = 0.550, Phi=0.237).

## Introduction

The development of bacteria that have become resistant to antibiotics can complicate the treatment process. One of the causes of antibiotic resistance is the inappropriate use of antibiotics. Some resistant bacteria that often appear include

methicillin-resistant *Staphylococcus epidermidis*, *Vancomycin-resistant Enterococci*, Gram-negative bacteria that are resistant to the *β-lactam* group (Desiyana, 2008).

The use of antibiotics that are not following existing resistance patterns can cause bacterial resistance to an antibiotic. One of the principles behind the emergence and spread of resistance between bacteria is the prevalence of resistance, which is directly proportional to the number of antibiotics used in various treatments. This is illustrated by the increase in antibiotic resistance in several countries that do not limit the use of antibiotics (Elliot *et al*., 2013). Therefore, it is necessary to make an effort to determine the suitability of using antibiotics based on the results of culture and bacterial sensitivity tests.

*Gentamycin* is an *Aminoglycoside* derivative antibiotic that is very significant, especially because of its role against Gram-negative bacteria. This compound is used for bacteria that are resistant to other antibiotics. The mechanism of *Gentamycin* action is by binding irreversibly to the 30S subunit of the bacterial ribosome which results in inhibiting protein synthesis and causing an incorrect translocation of the genetic code. *Gentamycin* is bactericidal. *Gentamycin* is effective against a wide range of Gram-negative bacterial strains including *Escherichia, Enterobacter, Klebsiella, Proteus,* and *Pseudomonas* species. For against Gram-positive microorganisms, *Gentamycin* is effective for *Staphylococcus aureus* and *Staphylococcus epidermidis*.

The research conducted by Winarto in 2004 - 2005, regarding the prevalence of ESBL (Extended Spectrum Beta-Lactamase) bacteria from blood specimens at Dr. Kariadi General Hospital, it was stated that the pattern of the effectiveness of *Gentamycin* antibiotics against ESBL bacteria was> 40%. The effectiveness of the antibiotic *Gentamycin* against various kinds of bacteria was varies, which against *Acinetobacter baumannii* by 40%, *E. coli* ESBL 63%, *Klebsiella aerogenes* 70%, *Klebsiella pneumoniae* ESBL 71.5%, and even against *Pseudomonas aeruginosa* by 92.5%, so the authors feel the need to changes research in the effectiveness of the antibiotic *Gentamycin* against *E. coli* ESBL.

The change in sensitivity to *Gentamycin* was influenced by the genes encoding *Aceltiltransferase* ααc (3) - IIα and ααc (3) - VIα which in the R. plasmid where if the bacteria were exposed to *Gentamycin* continuously then *Aceltyltransferase* ααc (3) - IIα and ααc (3) - VIα will be expressed by releasing the enzyme *N acetyltransferase*. Then the enzyme will influence the bacteria to

change its catch point in the 30S Ribosome sub-unit.

In the plasmid, several other resistant genes are likely to be ESBL coding genes, namely BlaTEM, BlaSHV, and CTX, so that if the ααc (3) - IIα and ααc (3) - VIα *Aceltyltransferase* genes in plasmids are expressed due to repeated exposure to *Gentamycin*, the ESBL gene will also expressed the bacteria changed, which initially was *Gentamycin* susceptible *E. coli* non-ESBL, which turned into *Gentamycin* resistant producing ESBL *E. coli*.

**Methods**

This research is an experimental study by providing treatment and observation of non-ESBL *E. coli* bacteria from urine culture. This study used a pre-post test only design, where the test was conducted at the beginning and the end of the study to see the changes that occurred after the treatment was carried out. The study of the population was clinical isolates of *E. coli* stored from urine specimens in the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya. The research sample was clinical isolate *E. coli* which stored from urine specimens and was susceptible to *Gentamycin* and non-ESBL in the Clinical Microbiology Unit of the Dr. Soetomo Surabaya Hospital from May to August 2019.

So for this study we used 30 isolate of susceptible Gentamicyn non-ESBL *E. coli*. The sample's inclusion criteria are *E.coli* isolate which susceptible to *Ceftazidime, Cefotaxime, Ceftriaxone,* and *Cefoperazone Sulbactam*; these bacterial isolates have been identified and tested for antimicrobial sensitivity using an automatic technique (Phoenix TM or Vitek 2); *E.coli* which susceptible to *Gentamycin.* And the exclusion criteria is stored *E.coli* isolates that do not grow.

A sampling of bacterial clinical isolates from urine specimens was carried out using a consecutive sampling technique. Each sample that meets the research criteria is taken so that the required sample size is met. The research was conducted at the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya. From May 2019 - August 2019. The main material that used in this research was non-ESBL *E. coli* bacterial, isolates stored from the Clinical Microbiology Unit of Dr. Soetomo Surabaya. Additional materials used in this study were Mueller-Hinton agar medium, 30 µg *Cefotaxime* antibiotic disc, and 10 µg *Gentamycin*.

**Result and Discussion**

The samples of this study were 30 *Escherichia coli* isolates obtained from clinical specimens taken by consecutive sampling. From May 2019 to August 2019

samples were collected. *E. coli* isolates that were susceptible to *Gentamycin* non ESBL obtained from the automatic phoenix machine were retested using the Kirby-Bauer method. The re-sensitivity test using the Kirby-Bauer antibiotic disk diffusion method was carried out to equate the method used during the ESBL screening and confirmation test and to the sensitivity of *Gentamycin.*

All isolates that met the inclusion-exclusion criteria were repeatedly exposed to *Gentamycin* (CN) discs for 14 days. The exposure was carried out every day in agar media containing MH agar using *Gentamycin* discs with a maximum length of study of 1-14 days. If within 1-14 days of the study, a positive result of *Gentamycin* resistance is obtained, then it will be continued by giving *Cefotaxime* exposure to screen for ESBL. At the beginning of the research, after the *E. coli* obtained from the automatic phoenix machine was then re-tested using the Kirby-Bauer method, it was found that 30 (100%) *E. coli* isolates were susceptible to *Gentamycin* and susceptible to *Cefotaxime*.

Then the 30 *E. coli* isolates were exposed to *Gentamycin* discs for 1-14 days. Every day, it was observed whether there were phenotypic changes in the *E. coli* isolate. Isolates that were still resistant to *Gentamycin* were replanted on MH agar media and then exposed to *Gentamycin* discs. This was done continuously for 14 days.

**Table 1. *E. coli* Resistance to Exposure of *Gentamicyn* 10ug with Kirby-Bauer Method (total n= 30)**

| No | Exposure | Exposure of *Gentamycin* 10µg | |
|---|---|---|---|
| | | **Sensitivity** | **Resistance** |
| 1 | Day - 2 | 1 (3,3%) | 29 (96,7%) |
| 2 | Day - 4 | 1 (3,3%) | 28(93,3%) |
| 3 | Day - 10 | 2 (6,7%) | 26(86,7%) |

From the research, it was found that the *E. coli* phenotype changes from *Gentamycin* susceptible to *Gentamycin* resistance. These changes occurred on day 2 of 1 isolate of *E. coli* (3.3%), day 4 of 1 isolate (3.3%), and day 10 of 2 isolates of *E. coli* (6.7%). The next stage is that *E. coli* which has undergone a phenotypic change is tested using a Cefotaxim disc as an ESBL screening.

**Table 2. *E. coli Gentamycin* Resistance to Exposure of *Cefotaxime* 30ug with Kirby-Bauer Method (total n= 30)**

| No | Exposure | Exposure of *Cefotaxim* 30 µg | |
|---|---|---|---|
| | | Sensitivity | Resistance |
| 1 | Day - 1 | 0 (0%) | 4 (100%) |

The results showed that 4 *E. coli* isolates that had changed their phenotypes to *Gentamycin*-resistant *E. coli* were also phenotypically changed to *E. coli* ESBL. Furthermore, after the completion of the study time of 14 days, 26 isolates of *E. coli* that were susceptible to *Gentamycin* were exposed to *Cefotaxim* discs as ESBL screening.

**Table 3. *E.coli suseptibel Gentamycin* Resistance to Exposure of *Cefotaxim* 30µg with Kirby-Bauer Method (total n=26)**

| No | Exposure | Paparan *Gentamycin* 10µg | |
|---|---|---|---|
| | | **Sensitivity** | **Resistance** |
| 1 | Day - 1 | 8 (30,8%) | 18 (69,2%) |

The results showed that 8 isolates of *E. coli* non-ESBL (30.8%) and 18 isolates of *E. coli* (69.2%) had phenotypic changes to *E. coli* ESBL. From the statistics, it was found that there was no significant relationship between *Gentamycin* susceptible *E. coli* non-ESBL and *Gentamycin* ESBL resistant *E.coli* (P = 0.550, Phi = 0.237).

In this study, 30 *E. coli* isolates were exposed to Gentamycin. The results showed that *E. coli* isolates had phenotypic changes from Gentamycin susceptible *E. coli* to Gentamycin resistant *E. coli*. This change occurred on day 2 in 1 isolate, day 4 in 1 isolate and day 10 in 2 isolates. So in total, 4 *E. coli* isolates that had phenotypic changes from Gentamycin susceptible *E. coli* to Gentamycin resistant *E. coli*.

This is related to the ability of bacteria to adapt to their environment. The presence of Gentamycin in the environment makes E.coli try to spread the gene coding for Gentamycin resistance via plasmids. Bacterial cells can respond to antibiotics so that they become resistant. These mechanisms include a decrease in the concentration of intracellular antibiotics of bacteria, changes in antibiotic molecules, and changes in antibiotic targets of action (Munita *et al.*, 2016).

In this study, the results showed that *E. coli* experienced a phenotypic change from Gentamycin susceptible *E. coli* to *Gentamycin* resistant *E. coli*. This is due to

the gene encoding the resistance code for *Gentamycin* Antibiotics, namely Aseltiltransferase ααc (3) - IIα and ααc (3) - VIα which are in plasmid R, if bacteria are exposed to *Gentamycin* continuously then Aseltiltransferase ααc (3) - IIα and ααc (3) ) - VIα will be expressed by releasing the enzyme N acetyltransferase. Then the enzyme will influence the bacteria to change their catch point in the 30S Ribosome sub-unit.

From the research, it was found that 4 *E. coli* isolates experienced phenotypic changes, but the rest remained susceptible to *Gentamycin* because the resistance mechanism of each antibiotic class could be different from other groups. Some researchers mention cross-resistance between different antibiotic classes (Talan et al., 2016). Furthermore, both *E. coli* isolates that were still susceptible to *Gentamycin* and those who had undergone phenotypic changes to be *Gentamycin* resistant were exposed to *Cefotaxime* as an ESBL screening. The results obtained on exposure to *Gentamycin* susceptible, *E. coli* with *Cefotaxime* obtained have the following results; 8 (30.8%) isolates that were still susceptible to *Cefotaxime* and 18 (69.2%) isolates that had turned into *Gentamycin* ESBL susceptible *E. coli*.

The results of 4 isolates *E. coli* resistant to *Gentamycin* (100%) after being exposed to *Cefotaxime*, all isolates were resistant to *Cefotaxime*. This is following research conducted by Amin (2017) from the Clinical Microbiology Unit of the Dr. Soetomo Surabaya Hospital, where in this study there was a change in phenotypic properties from 4 (25%) non-ESBL *E. coli* isolates to ESBL *E. coli* after being exposed to Ciprofloxacin. The phenotypic change from *Gentamycin* susceptible *E. coli* non-ESBL to *Gentamycin* ESBL resistant *E. coli* is due to exposure to a class of antibiotics, in this case *Gentamycin* can cause cross-resistance to other antibiotic classes, in this case, the beta-lactam group. Resistant strains can spread the resistant genes that are in their mobile gene to other bacteria horizontally, allowing viable bacteria that initially do not have a resistant gene to turn into a resistant strain. Conjugation is the most frequent gene transfer mechanism (Thacker D James et al., 2012). From the statistics, there was no significant relationship between *Gentamycin* susceptible *E. coli* non-ESBL and *Gentamycin* ESBL resistant *E. coli* after being exposed to *Gentamycin* disk for 14 days (P = 0.550, Phi = 0.237).

**Conclusion**

The conclusion that can be drawn from the results of this study is that. Repeated exposure to *Gentamycin* for 14 days was not statistically significant to cause changes in the phenotype of non-

ESBL *Gentamycin* susceptible Escherichia coli isolates to *Gentamycin*-resistant Escherichia coli isolates.

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